PERGAMON

Journal of Thermal Biology 25 (2000) 363–371

Journal of
THERMAL BIOLOGY

www.elsevier.com/locate/jtherbio

Heat shock proteins in whiteflies, an insect that accumulates sorbitol in response to heat stress

Michael E. Salvucci*, Dawn S. Stecher, Thomas J. Henneberry

US Department of Agriculture, Agricultural Research Service, Western Cotton Research Laboratory, 4135 E. Broadway Road, Phoenix, AZ, 85040-8830, USA

Received 22 July 1999; accepted 15 October 1999

Abstract

1. The heat shock response was examined in the silverleaf whitefly. Hsp70 and Hsp90 were the major polypeptides synthesized by whiteflies in response to heat stress.
2. The amounts of Hsp70 and Hsp60 protein and Hsp70 mRNA were unchanged in response to a diurnal increase in temperature, whereas sorbitol content increased eight-fold. That steady-state levels of Hsps did not increase with higher rates of synthesis suggests that Hsps turn over faster in heat-stressed whiteflies.
3. *Hsp70* transcript levels were highest when nutrient deprivation accompanied heat stress. Thus, Hsps appear to be especially important for heat-stressed whiteflies when sorbitol synthesis is limited nutritionally.

Keywords: Heat shock proteins; Hsp; *Bemisia*; Sorbitol; Polyols; Heat stress; Thermotolerance

1. Introduction

In the desert southwest of the US, silverleaf whiteflies, *Bemisia argentifolii*, Bellows and Perring, increase in population size during the summer (Henneberry et al., 1998) when air temperatures often exceed 42°C and relative humidities can be less than 10% (Brown and Russell, 1987). By feeding on the lower surface of leaves, whiteflies are protected to some extent from the temperature and humidity extremes of the environment. Still, the temperature of a mature leaf of a well-watered cotton plant can exceed 35°C (Lu et al., 1997), with even higher temperatures occurring on drought-stressed leaves (Radin et al., 1994).

Previous studies from this laboratory have shown that whiteflies and aphids, two very small phloem-feeding insects, accumulate polyhydric alcohols (polyols) in response to high temperature (Wolfe et al., 1998; Hendrix and Salvucci, 1998; Salvucci et al., 1999). Polyol synthesis in these insects is catalyzed by an NADPH-dependent ketose reductase (KR). For whiteflies, accumulation of sorbitol occurs under field conditions in response to a diurnal increase in temperature (Wolfe et al., 1998). Sorbitol and other polyols are well-known solvent modifiers that protect the native structure of

Abbreviations: Hsp, heat shock protein; KR, ketose reductase; DIG, digoxigenin; DTT, dithiothreitol; PMSF, phenylmethylsulfonyl fluoride.

* Corresponding author. Tel.: +1-602-379-3524; fax: +1-602-379-4509.

E-mail address: mesalvu@ix.netcom.com (M.E. Salvucci).

0306-4565/00/$ - see front matter
PII: S0306-4565(99)00108-4

proteins from thermal denaturation (Back et al., 1979; Henle et al., 1982). Recent evidence with whiteflies is consistent with the idea that sorbitol acts as a thermoprotectant in vivo, protecting whitefly proteins from thermal denaturation (Salvucci, 2000).

A common response to heat stress in all organisms, including insects (Denlinger and Yocum, 1998) is the synthesis of Hsps (reviewed in Parsell and Lindquist, 1993; Somero, 1995). These proteins are molecular chaperones that bind to and stabilize unfolded proteins (Hendrick and Hartl, 1993; Parsell and Lindquist, 1993). Hsps are distributed ubiquitously, occurring in both thermophilic (Trent et al., 1990) and psychrophilic (Deegenaars and Watson, 1998) organisms. For most organisms, Hsps are synthesized when ambient temperatures exceed the normal temperature optimum of the organism (Parsell and Lindquist, 1993). Studies with transgenic organisms, including *Drosophila* (Feder et al., 1996; Krebs and Feder, 1997b), have shown that Hsps improve thermal fitness (Parsell and Lindquist, 1993). Since whiteflies accumulate sorbitol in response to high natural temperatures (Wolfe et al., 1998), it was of interest to determine if the heat shock response of this insect was suppressed, particularly in response to the diurnal increase in temperature that these insects regularly encounter. Included in the present study were measurements of Hsp60, a prokaryotic chaperone that is a major protein synthesized by the endosymbiotic bacteria of aphids (Ishikawa, 1990).

2. Materials and methods

2.1. Heat stress under glasshouse conditions

Whiteflies were raised through at least five generations on cotton plants (*Gossypium hirsutum* L., cv. Coker 100A glandless) in either a "high temperature" or a "cooled" glasshouse. The "high temperature" glasshouse reached a maximum air temperature of 42°C, similar to the temperatures that occur in the desert southwest of the US during the summer months (Brown and Russell, 1987). The "cooled" glasshouse was limited to a maximum air temperature of 30°C. Whiteflies were collected in the early morning when leaf temperatures in both glasshouses were at their minimum (i.e., 25–27°C) and again at mid-day when leaf temperatures in the "cooled" and "high temperature" glasshouse were 28 and 36–39°C, respectively. Temperature was measured with a Type T needle thermocouple. Whiteflies were frozen immediately following collection and stored at −80°C for later analysis.

2.2. Heat stress under laboratory conditions

Whiteflies were placed on cotton plants at 25 and 40°C or in tubes without a source of nutrition at 40°C as described previously (Salvucci, 2000). Whiteflies were collected from the leaves and immediately frozen and stored at −80°C.

Drosophila melanogaster Meigen were kindly provided by Dr. Winifred Doane (Arizona State University). For heat shock experiments, approximately 25 adults were placed in an Erlenmeyer flask. The temperature of the flask was increased 1°C every 5 min from 25 to 37°C and then held at 37°C for 1 h. For an unstressed control, *Drosophila* were placed in a flask that was maintained at 25°C for 2 h. *Drosophila* were frozen immediately following each treatment and stored at −80°C.

2.3. Measurement of sorbitol content

The sorbitol content of whiteflies collected at various times of the day was determined as described previously (Wolfe et al., 1998; Salvucci, 2000).

2.4. Protein synthesis in vivo

Detached cotton leaves were labeled with ^{35}S for 3 h at low light (30 μmol photons m^{-2} s^{-1}) by placing the petiole in water containing 0.125 mCi of [^{35}S]Met/Cys (Amersham Life Science, Arlington Heights, IL)[1]. Whiteflies were acclimated to either 25 or 40°C by placing approximately 200 individuals in clip cages on unlabeled cotton leaves in the light (300 μmol photons m^{-2} s^{-1}). For acclimation and treatment at 40°C, whiteflies were maintained on an unlabeled leaf for 1 h at 25°C, and then the temperature was steadily increased to 40°C over an hour. Whiteflies were held at 40°C on the unlabeled leaf for 1 h and were then transferred to a labeled leaf for 2 h at 40°C. For acclimation and treatment at 25°C, whiteflies were maintained at 25°C for 3 h on an unlabeled leaf and were then transferred to a labeled leaf for 2 h at 25°C. In vivo labeling of whitefly proteins was also conducted with whiteflies from the high temperature and cooled glasshouses (see above) by transferring whiteflies at mid-day to a labeled leaf at 40 or 25°C, respectively.

To analyze labeled polypeptides, labeled whiteflies were collected by aspiration and immediately frozen at −80°C. Approximately 100 frozen whiteflies were extracted in 150 mM Mops-KOH, pH 7, containing 5 mM $MgCl_2$, 2 mM EDTA, 5 mM DTT, 1 mM PMSF and 20 μM leupeptin (extraction buffer). Cold

[1] Mention of a trademark, proprietary product, or vendor does not constitute a guarantee or warranty of the product by the US Department of Agriculture and does not imply its approval to the exclusion of other products or vendors that may also be suitable.

methanol was added to the homogenate to a final concentration of 80% (v/v). After incubation overnight at −20°C, precipitated protein was collect from the methanolic extracts by centrifugation for 10 min at 13,000 *g*. Precipitated protein was dried in vacuo, and suspended in buffer containing SDS (Salvucci et al., 1998). The suspension was heated for 2 min at 90°C, centrifuged for 1 min at 13,000 *g* and the supernatant was then electrophoresed in 7.5 to 15% SDS–PAGE gels (Salvucci et al., 1998). Following electrophoresis, polypeptides were transferred to PVDF membrane by electroblotting overnight at 100 mA (Salvucci et al., 1998). Membranes were rinsed in H_2O and the labeled polypeptides were visualized by direct autoradiography. The molecular weight of individual polypeptides was determined using an image acquisition densitometer calibrated with known molecular weight standards (Salvucci et al., 1992).

For two-dimensional electrophoresis, approximately 200 whiteflies were extracted as described above and total protein was precipitated with 80% methanol. The dried protein pellets were suspended in a solution containing 8 M urea, 1% SDS, 0.02% nonidet-40 and 0.01% 2-mercaptoethanol and separated by isoelectric focusing and SDS–PAGE as described previously (Duncan and Hershey, 1984). Gels were stained with Coomassie Blue followed by silver stain.

2.5. In vitro translation of whitefly mRNA

Total RNA was isolated from whiteflies by using the Triazol method as described by the manufacturer (Gibco BRL-LifeTechnologies, MD). Poly A^+ RNA was isolated using magnetic oligo dT beads (Dynabeads Oligo $(dT)_{25}$, Dynal Inc., Oslo, Norway) and 40 μg was translated in the presence of [^{35}S]Met/Cys using a wheat germ system (Amersham Life Sciences). Labeled polypeptides were separated by SDS–PAGE as described above.

2.6. Western blot analyses of protein expression

For Western blots, whiteflies were homogenized at 4°C in extraction buffer and the protein concentration of the solution was determined (Bradford, 1976). Aliquots of the homogenates containing 80 μg of protein were supplemented with acetone to 80% (v/v). After 1 h at −20°C, the extracts were centrifuged for 10 min at 13,000 *g*. The supernatants were discarded and the protein pellets were dried and then suspended in buffer containing SDS, dithiothreitol and sucrose (Salvucci et al., 1998). After heating to 100°C for 2 min, the extracts were centrifuged for 1 min and the supernatants were electrophoresed in 12% SDS–PAGE gels (Salvucci et al., 1998). Polypeptides were electrophoretically transferred to Immobilon-P PVDF membrane and the Hsp60, Hsp70 and KR polypeptides were detected immunologically using monospecific primary and alkaline phosphatase-conjugated secondary antibodies (Salvucci et al., 1998). Antibodies to KR have been described previously (Salvucci et al., 1998). Monoclonal antibodies to *Synechococcus* Hsp60 and chicken oviduct Hsp70 were obtained from StressGen Biotechnologies Corp. (Victoria, BC, Canada).

2.7. Northern blot analyses of RNA expression

Ten micrograms of total RNA, isolated as described above, was separated under denaturing conditions on formaldehyde-containing agarose gels (Sambrook et al., 1989). Following electrophoresis, the RNA was transferred to a nylon membrane (Boehringer Mannheim, Indianapolis, IN). Membranes were baked at 80°C for 1 h and then probed with a DIG-labeled oli-

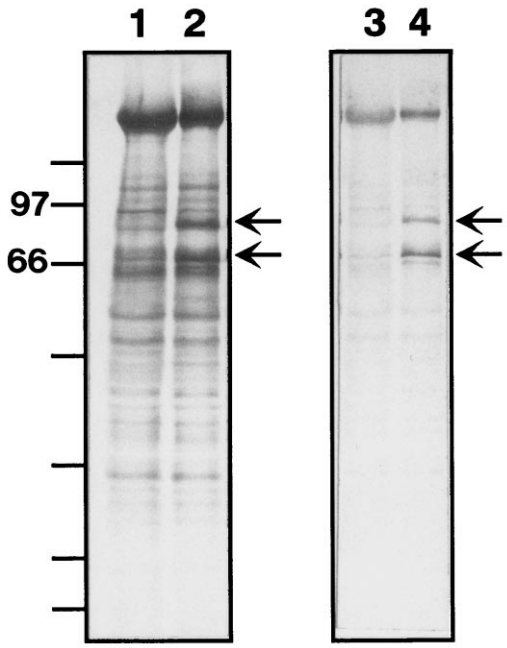

Fig. 1. In vivo labeling of whitefly Hsps with [^{35}S]Met/Cys. Whiteflies were acclimated on an unlabeled cotton leaf for 1 h at either 25 (lane 1) or 40°C (lane 2) and were then transferred to a labeled leaf at the same temperature. Whiteflies were collected from the cooled (lane 3) and the high temperature (lane 4) glasshouses and were transferred to a labeled leaf at 25 and 40°C, respectively. Following labeling, equal numbers of whiteflies were extracted and their proteins separated by SDS–PAGE. Labeled polypeptides were visualized by autoradiography. The arrows indicate the positions of Hsps. The lines to the left of the figure indicate the positions of standard proteins of 97 and 66 kDa (marked) and 116, 45, 31, 21 and 14 kDa (unmarked).

gonucleotide according to the manufacturer's instructions (Boehringer Mannheim, Indianapolis, IN).

A DIG-labeled riboprobe to *Hsp70* was synthesized using the Genius 4 Kit (Boehringer Mannheim, Indianapolis, IN). The template for riboprobe synthesis was a plasmid containing an insert that encodes for *Drosophila* Hsp70 EST GM05226 (Research Genetics Inc., Huntsville, AL). Transcripts for *Hsp70* were detected on blots using a chemiluminescence detection system after hybridizing with the DIG-labeled oligonucleotides as described by the manufacturer (Boehringer Mannheim, Indianapolis, IN). The relative amounts of each transcript were determined by whole-band analysis using an image acquisition densitometry (Salvucci et al., 1992).

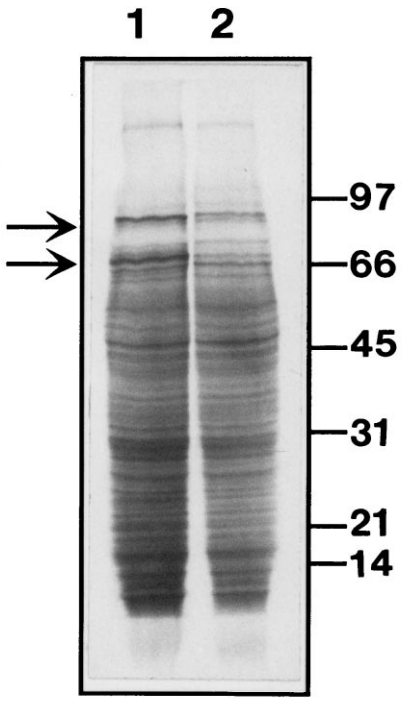

Fig. 2. In vitro translation of mRNA from unstressed and heat-stressed whiteflies. PolyA+ RNA was isolated from whiteflies that were acclimated on a cotton leaf for 1 h at either 40 (lane 1) or 25°C (lane 2) and 40 μg of each was translated in a wheat germ system. Labeled polypeptides were separated by SDS–PAGE and visualized by autoradiography. The arrows indicate the positions of Hsps. The numbers and lines to the right of the figure indicate the positions and molecular weights in kilodaltons of standard proteins.

3. Results

3.1. Synthesis of Hsps in whiteflies

The major difference in protein labeling between adult whiteflies labeled with [^{35}S]Met/Cys at 40 and 25°C was increased synthesis at 40°C of a 93 and a 73 kD polypeptide (Fig. 1). The molecular weights of these polypeptides corresponded to Hsp90 and Hsp70, two of the major Hsps (reviewed in Parsell and Lindquist, 1993). Increased synthesis of these two polypeptides at 40°C occurred when whiteflies were conditioned in the laboratory on cotton leaves for 1 h at 40°C prior to labeling as well as when whiteflies were transferred directly from the high temperature glasshouse to a labeled leaf without conditioning (Fig. 1). Whiteflies transferred from the high temperature glasshouse contained less total label than those conditioned on a leaf in the laboratory, indicating that the transfer from glasshouse to laboratory interrupted feeding.

RNA isolated from whiteflies exposed to 40 and 25°C produced similar profiles of labeled polypeptides when translated in vitro (Fig. 2). At both temperatures, the 93 and 73 kD Hsps appeared to be among the translation products. However, these polypeptides were significantly enriched among the translation products of mRNA from the 40°C whiteflies.

3.2. Sorbitol levels in whiteflies experiencing diurnal changes in temperature

Whiteflies maintained on cotton plants in a glass-

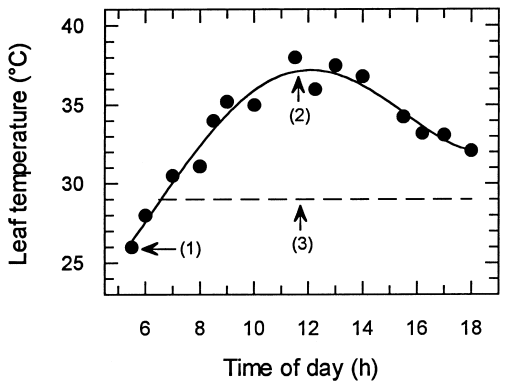

Fig. 3. Diurnal changes in leaf temperature of cotton plants in glasshouses set at two different temperatures. Leaf temperatures was measured during the course of a typical summer day in the high temperature (●) and cooled (— —) glasshouses. The arrows and numbers in parentheses indicate the various times that whiteflies were collected from the leaves (see Table 1).

Table 1
Leaf temperature and sorbitol content of whiteflies in the high temperature and cooled glasshouses at the times of sampling

Time of day[a] (a.m.)	Leaf temperature (°C)	Sorbitol content[b] (nmol whitefly^{-1})
6 (1)[c]	26	0.18 ± 0.09
11:40 (2)	37	1.56 ± 0.77
11:40 (3)	28	0.34 ± 0.11

[a] The numbers in parentheses refer to the numbered arrows in Fig. 3.
[b] Mean $\pm$ SEM of three separate samples, each consisting of 50–75 whiteflies.
[c] Leaf temperatures and sorbitol content at this sampling time were identical in the high temperature and cooled glasshouses.

house experienced diurnal changes in leaf temperature from a minimum of 26°C at 5:30 a.m. to a maximum near mid-day of 37–39°C in the high temperature glasshouse or 28°C in the cooled glasshouse (Fig. 3). For whiteflies in the high temperature glasshouse, sorbitol levels increased more than eight-fold from 5:30 to 11:40 a.m. (Table 1). In contrast, sorbitol levels increased less than two-fold over this same time period for whiteflies in the cooled glasshouse.

3.3. Levels of Hsp and KR protein in whiteflies experiencing diurnal changes in temperature

Commercially available antibodies to Hsp70 and Hsp60 recognized polypeptides of the appropriate apparent molecular weights on Western blots of whitefly polypeptides (Fig. 4). According to the supplier, the Hsp70 antibodies recognize both the constitutive and induced forms of Hsp70 in several mammalian species, as well as in frogs, chickens, yeast, *Drosophila* and tunicates. These antibodies recognized two polypeptides of about 72 and 70 kD on blots of whitefly polypeptides. Commercially available antibodies to Hsp90 did not recognize an Hsp90 cognate among whitefly polypeptides, consequently they were not used in the study (data not shown). The lack of recognition indicated that either commercial Hsp90 antibodies did not cross-react with whitefly Hsp90 or whiteflies do not contain an Hsp90 cognate.

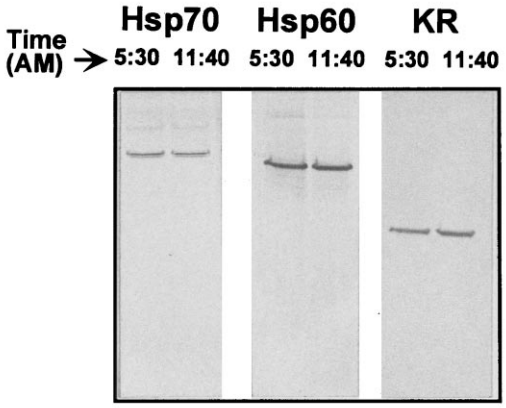

Fig. 4. Effect of diurnal temperature changes on the levels of Hsp70, Hsp60 and KR protein in whiteflies. Whiteflies were collected from the high temperature glasshouse at the indicated times and the proteins were analyzed on Western blots. Equal amounts of protein were loaded in each lane.

Whiteflies from the high temperature glasshouse had similar levels of Hsp70 and Hsp60 at 5:30 and 11:40 a.m. (Fig. 4), and there were no major differences in the level of the sorbitol-synthesizing protein, KR. Similarly, Hsp60, Hsp70 and KR protein levels were equivalent in whiteflies collected from the high temperature and cooled glasshouses at mid-day (Fig. 5). Whiteflies exposed to 25 and 40°C for 1 h on a cotton plant in the laboratory also had similar levels of Hsp70 (data not shown). Comparison of the polypeptide profiles of 25 and 40°C whiteflies by one- and two-dimensional electrophoresis showed that there was no difference in the amounts or distribution of the polypeptides detectable with Coomassie Blue or silver staining (data not shown).

3.4. Levels of Hsp70 mRNA in whiteflies experiencing diurnal changes in temperature

The levels of *Hsp70* transcripts were similar in the morning and at mid-day in whiteflies from the high temperature glasshouse (Fig. 6). In contrast, whiteflies feeding on cotton leaves in the laboratory had about ten-fold higher levels of *Hsp70* transcripts when exposed to 40 compared with 25°C. Whiteflies that were exposed to 40°C in the absence of a source of nutrition had even higher levels of *Hsp70*. Transcript levels for *Hsp70* were similar after feeding in the laboratory at 25, 33 and 36°C and only increased when the temperature was increased to 40°C (data not shown). *Hsp70* mRNA was almost undetectable in *Drosophila* adults exposed to 25°C, but increased to a very high level after exposure for 1 h to 37°C.

4. Discussion

In the present study, whiteflies raised in the high temperature glasshouse experienced air and leaf temperatures that were similar to those that are encountered naturally during the summer growing season in the desert southwest of the US (Brown and Russell, 1987; Lu et al., 1997). Under these conditions, the sor-

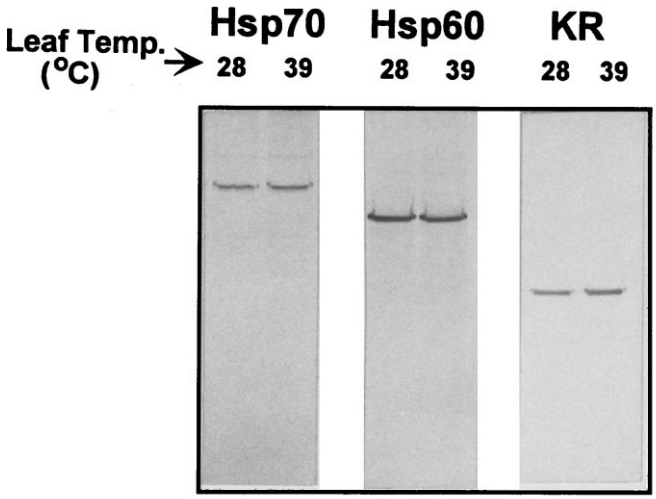

Fig. 5. Effect of temperature on the levels of Hsp70, Hsp60 and KR protein in whiteflies at noon. Whiteflies were collected at noon from cotton plants in the cooled (leaf temperature = 28°C) and the high temperature (leaf temperature = 39°C) glasshouse and the proteins were analyzed on Western blots. Equal amounts of protein were loaded in each lane.

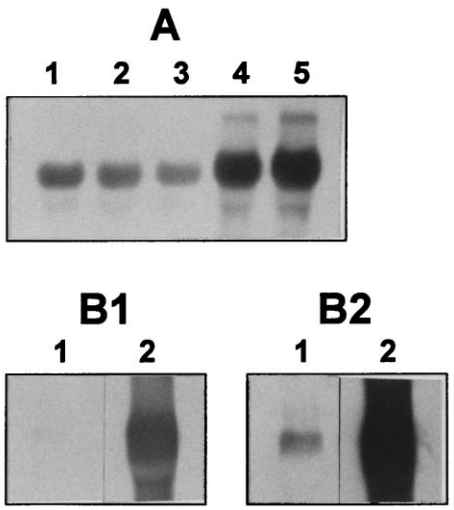

Fig. 6. Effect of temperature on the levels of *Hsp70* transcripts in whiteflies and *Drosophila*. Ten micrograms of total RNA from whiteflies (A) and *Drosophila* (B) were analyzed on Northern blots using a probe prepared to *Drosophila Hsp70*. (A) Whiteflies were collected from cotton plants in the high temperature glasshouse in the morning (lane 1) and at mid-day (lane 2) when leaf temperatures were 25 and 36°C, respectively. In a separate experiment, whiteflies were exposed to 25 (lane 3) or 40°C (lane 4) for 1 h in the laboratory while feeding on a cotton plant. Whiteflies were also incubated for 1 h at 40°C in the absence of a source nutrition (lane 5). (B) Adult *Drosophila* were incubated for 1 h at 25 (lane 1) and 37°C (lane 2). Panel B1 and B2 are two exposures of the same blot.

bitol content of the whiteflies increased eight-fold in response to a diurnal increase in leaf temperature from 26 to 38°C. In contrast, the sorbitol content of the whiteflies increased only two-fold when leaf temperatures increased from 26°C in the morning to only 28°C at mid-day. Thus, temperature appears to be the major factor responsible for diurnal changes in sorbitol content of whiteflies (Wolfe et al., 1998; Salvucci et al., 1999).

In vivo labeling of whitefly proteins at 25 and 40°C showed that the rate of synthesis of Hsp70 and Hsp90 increased at the higher temperature. However, there was no increase in the steady-state amount of Hsp70 in whiteflies exposed to elevated temperatures. Wood et al. (1998) reported a similar discrepancy in heat-shocked lampreys, suggesting that either the masking of inducible Hsp70 by constitutive Hsp70 or a lack of antibody recognition could explain why increased synthesis of Hsp70 was not accompanied by higher amounts of protein on Western blots. These explanations do not appear to be valid for whiteflies since Hsp70 antibodies recognized multiple forms of Hsp70 on Western blots of whitefly polypeptides. Also, separation of whitefly proteins by one- or two-dimensional gel electrophoresis gave no indication of an increase in the amounts of Hsps in response to heat stress. Thus, the turnover rate of Hsp70 in whiteflies must be faster at higher temperatures since heat stress increased the rate of synthesis (Fig. 1), but did not affect the steady-state level of protein. This conclusion does not preclude the likely possibility (Joplin and Denlinger, 1990; Krebs and Feder, 1997a) that the amount of Hsp70 in

whiteflies increases in response to heat shock, but that the increase occurs in certain tissues, without a noticeable effect on the total Hsp70 pool.

In contrast to the results with whiteflies, the amount of Hsp70 protein in both the larval and the adult forms of *Drosophila* increases nearly a 1000-fold in response to heat shock (Velazquez et al., 1983). Interestingly, Hsp70 is barely detectable in unstressed *Drosophila* (Velazquez et al., 1983; Krebs and Feder, 1997b; Feder et al., 1996), whereas unstressed whiteflies contain amounts of Hsp70 that are comparable to the amount present after heat stress. Similarly, Gehring and Wehner (1995) showed that significant levels of Hsp70 and Hsp72 occur in temperate and desert ant species prior to heat stress. These investigators proposed that the accumulation of Hsps prior to exposure to high temperature preadapts the ants to heat stress. The occurrence of high constitutive levels of Hsp70 in unstressed whiteflies appears to be an inherent characteristic rather than one that is induced by daily exposure to high temperature since whiteflies that were raised in a cooler glasshouse also had high levels of Hsp70.

In *Drosophila*, transcript levels for *Hsp70* increase nearly 1000-fold in response to heat stress (Velazquez et al., 1983; Goto et al., 1998). In the present study, transcript levels for *Hsp70* increased in heat-stressed compared with unstressed whiteflies. However, the increase was only about 10-fold and occurred in the laboratory when leaf temperatures reached 40°C, but not in the glasshouse when the leaf temperature increased from 26 to 36°C. Interestingly, the levels of *Hsp70* transcripts were relatively high in unstressed whiteflies, in contrast to unstressed *Drosophila* which contain levels that are barely detectable. The characteristics of *Hsp70* expression in whiteflies was similar to the pattern in *Ceratitis capitata* larvae where transcript levels for *Hsp70* increased only two-fold upon heat shock (Thanaphum and Haymer, 1998).

An Hsp60 homologue called symbionin is the major protein synthesized by the endosymbiotic bacteria in the pea aphid, *Acyrthosiphon pisum* (Ishikawa, 1990). Studies with this aphid have shown that synthesis of Hsp60 or possibly a phosphorylated form of this protein increases with heat stress (Morioko and Ishikawa, 1992). However, other investigators have shown that *Buchnera*, the endosymbiont in the aphid *Schizaphis graminum* contain high steady state levels of Hsp60 that are unaffected by a 10°C increase in temperature (Baumann et al., 1996). In the present study, Hsp60 was detected in whiteflies, but both its steady-state amount and its rate of synthesis were unaffected by temperature. Recent investigations with whiteflies (Morin et al., 1999) and earlier investigations with aphids (van den Heuvel et al., 1994) have shown that Hsp60 binds to virus particles and this binding appears to protect the virus from degradation during its passage from the hemocoel. Thus, Hsp60 appears to be required for aspects of the symbiosis unrelated to heat stress, perhaps explaining its presence in unstressed whiteflies and aphid.

In a previous study, an analogy was drawn between polyol synthesis in whiteflies and aphids and trehalose synthesis in yeast (Salvucci, 2000). The relationship between Hsps and trehalose in yeast has been characterized using mutants in both Hsp and trehalose synthesis (Ribeiro et al., 1997; Gross and Watson, 1998; Elliott et al., 1996; De Virgilio et al., 1994). The consensus view appears to be that both trehalose and Hsps contribute to thermotolerance, the former ameliorates long term heat stress while the latter are required for short term repair during heat shock (Gross and Watson, 1998). During the summer months, whiteflies often experience regular diurnal increases in air and leaf temperatures, sometimes exceeding 45 and 35°C, respectively (Brown and Russell, 1987; Lu et al., 1997). Under field conditions, whiteflies synthesize sorbitol that probably functions as a thermoprotectant to protect the insect during the diurnal exposure to high temperature (Wolfe et al., 1998).

A faster rate of synthesis and turnover of Hsp70 and Hsp90 and relatively high constitutive levels of these proteins may also provide some protection during the hottest times of the day. Previously, we showed that whiteflies do not accumulate sorbitol in the absence of a source of carbohydrates or when their nutritional source lacked sufficient carbohydrate (Salvucci, 2000). Here we show that transcript levels for *Hsp70* were highest when nutrient deprivation accompanied heat stress. Thus, even though whiteflies accumulate sorbitol as a thermoprotectant, there may be times when sorbitol levels are inadequate and Hsps provide the main protection against heat damage.

Acknowledgements

We would like to acknowledge the expert technical assistance of C. Chamberlain. We would also like to thank Dr. D.L. Hendrix and T. Steele for providing HPLC analyses of whitefly carbohydrates. The research was supported in part by grant No. 97-431 from Cotton Inc.

References

Back, J.F., Oakenfull, D., Smith, M.B., 1979. Increased thermal stability of proteins in the presence of sugars and polyols. Biochemistry 18, 5191–5196.

Baumann, P., Baumann, L., Clark, M.A., 1996. Levels of

Buchnera aphidicola chaperonin GroEL during growth of the aphid *Schizaphis graminum*. Curr. Microbiol. 32, 279–285.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of proteins utilizing the principle of protein-dye binding. Anal. Biochem. 72, 248–259.

Brown, P., Russell, B. 1987. Weather Data and On-Line Documentation. The Arizona Meteorological Network (AZMET) Computer Bulletin Board. The University of Arizona Cooperative Extension Service, Tucson, AZ (http://ag.arizona.edu/azmet/).

Deegenaars, M.L., Watson, K., 1998. Heat shock response in psychrophilic and psychrotrophic yeast from Antarctica. Extremophiles 2, 41–49.

Denlinger, D.L., Yocum, G.D., 1998. Physiology of heat sensitivity. In: Hallman, G.J., Denlinger, D.L. (Eds.), Temperature Sensitivity in Insects and Application in Integrated Pest Management. Westview Press, Oxford, pp. 7–53.

De Virgilio, C., Hottiger, T., Dominguez, J., Boller, T., Wiemken, A., 1994. The role of trehalose synthesis for acquisition of thermotolerance in yeast I. Genetic evidence that trehalose is a thermoprotectant. Eur. J. Biochem. 219, 179–186.

Duncan, R., Hershey, J.W.B., 1984. Evaluation of isoelectric focusing running conditions during two-dimensional isoelectric focusing/sodium dodecylsulfate-polyacrylamide gel electrophoresis: variations in gel patterns with changing conditions and optimized isoelectric focusing conditions. Anal. Biochem. 138, 144–155.

Elliott, B., Haltiwanger, R.S., Futcher, B., 1996. Synergy between trehalose and Hsp104 for thermotolerance in *Saccharomyces cerevisiae*. Genetics 144, 923–933.

Feder, M.E., Cartaño, N.V., Milos, L., Krebs, R.A., Lindquist, S.L., 1996. Effect of engineering *Hsp70* copy number on Hsp70 expression and tolerance of ecologically relevant heat shock in larvae and pupae of *Drosophila melanogaster*. J. Exp. Biol. 199, 1837–1844.

Gehring, W.J., Wehner, R., 1995. Heat shock protein synthesis and thermotolerance in *Cataglyphis*, an ant from the Sahara desert. Proc. Natl Acad. Sci. USA 92, 2994–2998.

Goto, S.G., Yoshida, K.M., Kimura, M.T., 1998. Accumulation of *Hsp70* mRNA under environmental stresses in diapausing and nondiapausing adults of *Drosophila triauraria*. J. Insect Physiol. 44, 1009–1015.

Gross, C., Watson, K., 1998. De novo protein synthesis is essential for the thermotolerance acquisition in a *Saccharomyces cerevisiae* trehalose synthase mutant. Biochem. Molec. Biol. Int. 45, 663–671.

Hendrick, J.P., Hartl, F.-U., 1993. Molecular chaperone functions of heat-shock proteins. Annu. Rev. Biochem. 62, 349–384.

Hendrix, D.L., Salvucci, M.E., 1998. Polyol metabolism in homopterans at high temperature: accumulation of mannitol in aphids (Aphididae: Homoptera) and sorbitol in whiteflies (Aleyrodidae: Homoptera). Comp. Biochem. Physiol. 120, 487–494.

Henle, K.J., Nagle, W.A., Moss Jr, A.J., Herman, T.S., 1982. Polyhydroxy compounds and thermotolerance: a proposed concatenation. Radiat. Res. 92, 445–451.

Henneberry, T.J., Jech, L.F., Hendrix, D.L., 1998. Seasonal distribution of *Bemisia argentifolii* (Homoptera: Aleyrodidae) honeydew sugars on Pima and upland cotton lint and lint stickiness at harvest. Southwest Entomol. 23, 105–121.

van den Heuvel, J.F.J.M., Verbeek, M., van der Wilk, F., 1994. Endosymbiotic bacteria associated with circulative transmission of potato leaf roll virus by *Myzus persicae*. J. Gen. Virol. 75, 2559–2565.

Ishikawa, H., 1990. Molecular interactions of intracellular symbionts and aphids. In: Campbell, R.K., Eikenbary, R.D. (Eds.), Aphid-Plant Interactions. Elsevier, Amsterdam, pp. 351–361.

Joplin, K.H., Denlinger, D.L., 1990. Developmental and tissue specific control of the heat shock induced 70 kDa related proteins in the flesh fly, *Sarcophaga crassipalpis*. J. Insect Physiol. 36, 239–249.

Krebs, R.A., Feder, M.E., 1997a. Natural variation in the expression of the heat-shock protein Hsp70 in a population of *Drosophila melanogaster* and its correlation with tolerance of ecologically relevant thermal stress. Evolution 51, 173–179.

Krebs, R.A., Feder, M.E., 1997b. Tissue-specific variation in Hsp70 expression and thermal damage in *Drosophila melanogaster* larvae. J. Exp. Biol. 200, 2007–2015.

Lu, Z., Chen, J., Percy, R.G., Zeiger, E., 1997. Photosynthetic rate, stomatal conductance and leaf area in two cotton species (*Gossypium barbadense* and *Gossypium hirsutum*) and their relation with heat resistance and yield. Aust. J. Plant Physiol. 24, 693–700.

Morin, S., Ghanim, M., Zeidan, M., Czosnek, H., Verbeek, M., van den Heuvel, J.F.J.M., 1999. A GroEL homologue from endosymbiotic bacteria of the whitefly *Bemisia tabaci* is implicated in the circulative transmission of tomato yellow leaf curl virus. Virology 256, 75–84.

Morioko, M., Ishikawa, H., 1992. Mutualism based on stress: selective synthesis and phosphorylation of a stress protein by an intracellular symbiont. J. Biochem. 111, 431–435.

Parsell, D.A., Lindquist, S., 1993. The function of heat-shock proteins in stress tolerance: degradation and reactivation of damaged proteins. Annu. Rev. Genet. 27, 437–496.

Radin, J.W., Lu, Z., Percy, R.G., Zeiger, E., 1994. Genetic variability for stomatal conductance in Pima cotton and its relation to improvements of heat adaptation. Proc. Natl Acad. Sci. USA 91, 7217–7221.

Ribeiro, M.J., Reinders, A., Boller, T., Wiemken, A., De Virgilio, C., 1997. Trehalose synthesis is important for the acquisition of thermotolerance in *Schizosaccharomyces pombe*. Molec. Microbiol. 25, 571–581.

Salvucci, M.E., 2000 Sorbitol accumulation in whiteflies: evidence for a role in protecting proteins during heat stress, J. Therm Biol. 25, 353–361.

Salvucci, M.E., Chavan, A.J., Haley, B.E., 1992. Identification of peptides from the adenine binding domains of ATP and AMP in adenylate kinase: isolation of photoaffinity-labeled peptides by metal chelate chromatography. Biochemistry 31, 4479–4487.

Salvucci, M.E., Wolfe, G.H., Hendrix, D.L., 1998. Purification and properties of an unusual NADPH-dependent ketose reductase from the silverleaf whitefly. Insect Biochem. Molec. Biol. 28, 357–363.

Salvucci, M.E., Hendrix, D.H., Wolfe, G.R., 1999. Effect of high temperature on the metabolic processes affecting sorbitol synthesis in the silverleaf whitefly, *Bemisia argentifolii*. J. Insect Physiol. 45, 21–27.

Sambrook, J., Fritsch, E.F., Maniatis, T., 1989. Molecular Cloning, A laboratory manual, 2nd ed. Cold Spring Harbor Laboratory, New York.

Somero, G.N., 1995. Proteins and temperature. Annu. Rev. Physiol. 57, 43–68.

Thanaphum, S., Haymer, D.S., 1998. A member of the hsp70 gene family from the Mediterranean fruit fly, *Ceratitis capitata*. Insect Molec. Biol. 7, 63–72.

Trent, J.D., Osipiuk, J., Pinkau, T., 1990. Acquired thermotolerance and heat shock in the extremely thermophilic archaebacterium *Sulfolobus* sp. Strain B12. J. Bacteriol. 172, 1478–1484.

Velazquez, J.M., Sonoda, S., Bugaisky, G., Lindquist, S., 1983. Is the major *Drosophila* heat shock protein present in cells that have not been heat shocked? J. Cell Biol. 96, 286–290.

Wolfe, G.R., Hendrix, D.L., Salvucci, M.E., 1998. A thermoprotective role for sorbitol in the silverleaf whitefly. J. Insect Physiol. 44, 597–603.

Wood, L.A., Brown, I.R., Youson, J.H., 1998. Characterization of the heat shock response in the gills of sea lampreys and a brook lamprey at different intervals of their life cycles. Comp. Biochem. Physiol. A120, 509–518.